TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2016) 40: 788-796
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doi:10.3906/vet-1512-54

Protective effects of S-adenosylmethionine (SAMe) and silybin on hepatorenal and hemostatic functions in dogs with endotoxemia

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Received: 16.12.2015 • **Accepted/Published Online:** 23.05.2016 • **Final Version:** 15.12.2016

Abstract: We aimed to investigate the protective effects of S-adenosylmethionine (SAMe) and silybin treatment on hepatorenal and hemostatic functions in dogs with endotoxemia. Healthy dogs (n = 20) were divided equally into 4 groups as control, SAMe + silybin (SS), lipopolysaccharide (LPS), and LPS + SS. Controls received 0.9% NaCl intravenously. Animals were treated daily with 20 mg of SAMe and 1 mg of silybin per kilogram of body weight in SS and LPS + SS. LPS was injected (2 µg/kg, once intravenously) to dogs in LPS and LPS + SS. Hemostatic functions were assessed by thromboelastography (TEG). LPS-induced clinical and hematological changes were attenuated ($P < 0.05$) by SS treatment. Serum blood urea nitrogen and creatinine increased at 1–24 h ($P < 0.05$) after LPS. They did not changed in LPS + SS. LPS associated increases in liver enzymes were inhibited at 1–24 h in LPS + SS. In TEG, reaction time, coagulation time, and α-angle, but not maximum amplitude and G values, were significantly changed after LPS. These responses were restored by SS treatment in dogs with LPS. SS administration may be beneficial by regulating coagulation and protecting hepatorenal function in dogs with endotoxemia.

Key words: S-Adenosylmethionine, silybin, endotoxemia, thromboelastography, dogs

1. Introduction

Canine sepsis is associated with substantial morbidity and mortality in veterinary medicine. Endotoxin entry into circulation from gram-negative bacteria, which are most commonly isolated in sepsis, gives rise to multiple organ failure, severe sepsis, and endotoxemia (1). Thus, experimental intravenous administrations of endotoxin (lipopolysaccharide, LPS) in humans (2), dogs (3–5), ovines (6), and calves (7) have been done to emulate sepsis (7,8). Hepatorenal injury and coagulation abnormalities such as disseminated intravascular coagulation (DIC) are the most common complications during sepsis (5,8,9).

Thromboelastography (TEG), a point-of-care hemostatic analyzer, evaluates the changes in the viscoelastic properties of whole blood from initial clot formation through fibrinolysis, thereby providing global assessment of the hemostatic process. TEG (kaolin-activated) has been validated for use in veterinary medicine, especially for dogs (9,10). As compared with the traditional methods [active coagulation time (ACT), prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer, etc.], TEG has been accepted as a sensitive and useful tool for evaluating an animal with hypercoagulopathy or hypocoagulapathy. TEG results are also better to predict thrombotic as well as hemorrhagic events in a clinical setting (11).

Parameters routinely evaluated on a TEG tracing included reaction time (R), coagulation time (K), maximum amplitude (MA), alpha angle (α-angle), and G value. The R value is an initiation time (time 0–2 mm amplitude) and primarily influenced by plasma clotting factors and natural anticoagulants. The K value evaluates clot kinetics (time 2–20 mm amplitude) and it is influenced by coagulation factors, fibrinogen plasma concentration, and platelet count. The α-angle, which evaluates clot kinetics with the slope between 2 and 20 mm amplitude, reflects the speed of fibrin cross-linking, and it is generally influenced by plasma fibrin concentration and less so by platelet function. The MA value is the measurement of the peak rigidity manifested by the clot. The G value is more indicative of small changes in clot rigidity or fibrinolysis (10,11).

In a recent study (12), a significant positive correlation between R time and PT or aPTT was reported. Studies also showed that fibrinogen concentration and platelet counts were correlated with G/MA and K time in dogs

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(11,12). Previous studies regarding the use of TEG showed hypercoagulability (evidenced by an increased MA) in dogs with parvoviral enteritis (13), neoplasia (14), or immune-mediated hemolytic anemia (15), and hypocoagulapathy (evidenced by increased R and K, and decreased α-angle and MA) in dogs with DIC (12). Reference ranges for TEG parameters in healthy dogs were reported as follows: R, 1.8–8.6 min; K, 1.3–5.7 min; α-angle, 36–74 degrees; MA, 42–67 mm; and G value, 3.2–9.6 kdyn/cm^2 (10).

The liver plays an important role in controlling systemic inflammatory response syndrome by producing acute-phase reactants, and in regulating the coagulation cascade by producing coagulant proteins (FI, II, V, etc.) and anticoagulant proteins (protein S, protein C, antithrombin). Thus, liver protection in the course of sepsis is crucial to decrease mortality (16,17). Recent studies showed that S-adenosylmethionine (SAMe) and silymarin could prevent liver injury in different clinical conditions (toxin-induced hepatopathy and alcohol-induced hepatopathy) (17) by regulating cytokine responses to the primary insult (18). SAMe is a compound that functions widely in the body as a group donor or enzymatic inducer during transmethylation, transsulfuration, and aminopropylation (17). The liver synthesizes the majority of SAMe in the body (17), and SAMe has been shown to be an important compound for liver health (17–19).

Silybin is an active part of an extract from milk thistle, known as silymarin, and has been shown to support liver function by helping maintain a healthy oxidative balance (17,20). Although silymarin was reported to improve the rate of survival of LPS-treated mice (20), there is no evidence yet to support the use of SAMe, silybin, or both in dogs with endotoxemia. Thus, in this study, the possible protective effects of SAMe and silybin treatment on hepatorenal and hemostatic functions in dogs with endotoxemia were investigated.

2. Materials and methods

2.1. Experimental animals and study design

Twenty adult mongrel dogs (20 males), housed in the animal hospital (Faculty of Veterinary Medicine, Uludağ University), were used in the study. The dogs weighed between 15 and 26 kg (mean ± SD: 20.5 ± 5.8 kg) and the ages of the dogs ranged from 2 to 4 years (mean ± SD: 2.6 ± 0.8 years). The dogs were clinically healthy based on normal clinical examinations and normal complete blood count results. This experimental protocol was approved by the Animal Care and Use Committee of Uludağ University, Bursa, Turkey (Approval ID: 2009 – 01 / 08).

The dogs were divided randomly into 4 equal groups: Control, SAMe + silybin (SS), lipopolysaccharide (LPS), and LPS + SS. Dogs in the control group received 5 mL of sterile saline solution intravenously. A single oral dose (20 mg/kg) of Denamarin (225 mg of SAMe + 24 mg of silybin in one tablet, Nutramax, USA) was administered in the SS group, and 5 min after LPS administration in the LPS + SS group. Dogs in the LPS and LPS + SS groups received endotoxin (lipopolysaccharide, *Escherichia coli* serotype 055:B5, purity >97%; Sigma, USA) dissolved in sterile saline (0.9% NaCl, solution; Baxter, İstanbul, Turkey) and administered intravenously at a sublethal dosage (2 µg/kg, intravenously, once), as described previously (3,21). Dogs were provided water 3 times a day and food (a pelleted diet) twice a day during the experiments. Dogs were monitored clinically and clinicopathologically for 24 h.

2.2. Sample collection and measurements

Blood samples were collected before treatment (0 h) and 1, 4, and 24 h after treatment from the brachiocephalic vein into vacutainer tubes containing K_2EDTA (BD Vacutainer System, BD Diagnostics, USA) for a complete blood count (CBC) and containing 3.2% sodium citrate (BD Vacutainer System, BD Diagnostics) for hemostatic evaluations. CBC and serum biochemistry analyses were performed just after the blood collection using automatic analyzers (HM5 and VetScan, Abaxis, UK). White blood cell (WBC) count, hematocrit, platelet count, serum liver enzyme activities (alanine aminotransferase [ALT] and alkaline phosphatase [ALP]), and renal injury markers (blood urea nitrogen [BUN] and creatinine [Cr]) were monitored in this study. Hemostatic functions were assessed by use of TEG (TEG-5000, Hemoscope, USA), as described previously (9,10,22). Five parameters of TEG were included for this study: reaction time (R), coagulation time (K), α-angle, maximum amplitude (MA), and G value.

2.3. Statistical analysis

Results were expressed as mean ± SD. The results for each parameter were evaluated for approximate normality of distribution using Kolmogorov–Smirnov statistics (SigmaStat version 3.0, Systat Software, USA). Data were evaluated by one-way or two-way analysis of variance (ANOVA) with repeated measures for within-group and between-group changes. $P < 0.05$ was considered significant.

3. Results

Endotoxin caused clinical findings (fever, tachycardia, tachypnea, prolonged capillary refill time, decreased peripheral pulse quality, depression, anorexia, vomiting, and diarrhea) and hematological responses (increased hematocrit level, leukopenia, and thrombocytopenia) within 1 h after injection. Selected parameters (body temperature, respiratory rate, and heart rate) are shown in Table 1. Some clinical (Table 1) and hematological abnormalities (Table 2) in response to LPS were inhibited ($P < 0.05$) or their severity was attenuated by SAMe and silybin treatment. Baseline WBC counts decreased at 1 h

Table 1. Selected clinical findings before (baseline) and after the treatments in all groups, each of which consisted of five dogs.

Parameters	Baseline	1 h	4 h	24 h
Temperature, °C				
Control	38.9 ± 0.4 ^{aA}	38.8 ± 0.4 ^{aA}	38.5 ± 0.2^{aA}	38.9 ± 0.4 ^{aA}
SS	38.1 ± 0.0 ^{aA}	38.5 ± 0.4 ^{aA}	38.5 ± 0.0^{aA}	38.4 ± 0.1 ^{aA}
LPS	38.9 ± 0.6 ^{aA}	39.8 ± 1.2^{bA}	39.8 ± 0.8^{bB}	39.1 ± 0.2^{abA}
LPS + SS	38.3 ± 0.0 ^{aA}	38.2 ± 0.1 ^{aA}	39.9 ± 0.3^{bB}	38.3 ± 0.2^{aA}
Respiratory rate, bpm				
Control	20 ± 4 ^{aA}	28 ± 6 ^{aA}	30 ± 4 ^{aA}	30 ± 2 ^{aA}
SS	30 ± 2 ^{aA}	28 ± 4 ^{aA}	27 ± 5 ^{aA}	28 ± 3 ^{aA}
LPS	29 ± 6^{aA}	46 ± 16^{bB}	36 ± 12^{abAB}	53 ± 28^{abB}
LPS + SS	28 ± 4^{aA}	44 ± 14^{bB}	43 ± 7^{bB}	25 ± 6^{aAB}
Heart rate, bpm				
Control	92 ± 10^{aA}	94 ± 14^{aA}	92 ± 16^{aA}	96 ± 6^{aA}
SS	110 ± 19^{aA}	90 ± 8^{aA}	92 ± 7^{aA}	98 ± 8^{aA}
LPS	104 ± 16^{aA}	136 ± 18^{bB}	102 ± 30^{abA}	126 ± 14^{abA}
LPS + SS	91 ± 12^{aA}	98 ± 8^{aA}	120 ± 10^{bA}	118 ± 7^{abA}

Control: Healthy dogs, SS: SAMe and silybin, LPS: lipopolysaccharide, LPS + SS: lipopolysaccharide + SAMe and silybin.
a, b: Different letters show statistically significant changes within groups, at least $P < 0.05$.
A, B: Different letters show statistically significant changes between groups, $P < 0.05 - P < 0.01$.

and then increased at 24 h in dogs receiving LPS (Table 2). The severities of leukocyte responses in the LPS and LPS + SS groups were similar to each other.

Serum levels of BUN and Cr increased at 1–24 h ($P < 0.05$) after LPS administration, but their levels did not change significantly for 24 h in the LPS + SS group (Table 3). LPS-associated increases in ALT and ALP activities ($P < 0.05$) were inhibited at 1–24 h in the LPS + SS group.

Of the TEG parameters, following LPS administration, R and K times were prolonged ($P < 0.05$) whereas the α-angle was decreased ($P < 0.01$) within 1 h (Figures 1A and 1B). MA and G values did not change significantly for 24 h in dogs receiving LPS. SAMe + silybin treatment attenuated these coagulation responses in dogs with LPS (Table 4). All dogs survived to the end of the study.

4. Discussion

This study showed that SAMe and silybin in combination might have a protective effect on hepatorenal and hemostatic functions in dogs with endotoxemia. Experimental canine endotoxemia used in the present study could provide a reliable setting to reflect the clinical, hematological, and serum biochemistry findings in human (2) and animal (3,8,23) cases of naturally occurring sepsis.

In this study, endotoxemia was characterized by pyrexia, tachycardia, tachypnea (Table 1), leukopenia, and thrombocytopenia (Table 2), as well as organ damage (Table 3) within 1 h after LPS administration in dogs, as reported in our previous experiments (3–5,9,21). These observations after LPS administration were likely due to activation of the initial endocrine-metabolic stress response, defined as acute-phase reaction (24), in which TNF-α is one of the major cytokines to regulate host immunologic reaction against LPS (8,16,19).

In this study, SAMe and silybin administration treated some clinical and hematological abnormalities in response to LPS. Observed WBC counts (leukopenia and then leukocytosis) in dogs with LPS were similar to those in dogs with LPS + SS, indicating the inability of SAMe and silybin treatment to control leukocyte responses to LPS. Recently, serum C-reactive protein (CRP), a positive acute-phase protein, has been used to indicate the presence of inflammatory reaction in dogs, and it was found better to predict systemic or local inflammation compared to some clinical and hematological findings such as body temperature, heart rate, WBC and platelet

Table 2. Selected hematological findings before (baseline) and after the treatments in all groups, each of which consisted of five dogs.

Parameters	Baseline	1 h	4 h	24 h
WBC count, $\times 10^3$/mL				
Control	11.1 ± 1.8^{aA}	10.8 ± 1.4^{aA}	12.3 ± 1.4^{aA}	11.8 ± 1.9^{aA}
SS	11.6 ± 3.9^{aA}	12.4 ± 2.7^{aA}	11.4 ± 1.6^{aA}	10.8 ± 3.9^{aA}
LPS	10.5 ± 4.4^{aA}	2.5 ± 2.3^{bB}	10.5 ± 4.2^{aA}	33.0 ± 11.8^{cB}
LPS + SS	10.1 ± 1.6^{aA}	2.3 ± 0.2^{bB}	6.7 ± 0.9^{aA}	31.3 ± 0.3^{cB}
Hematocrit, %				
Control	41 ± 5^{aA}	42 ± 3^{aA}	43 ± 3^{aA}	41 ± 2^{aA}
SS	44 ± 2^{aA}	43 ± 4^{aA}	43 ± 4^{aA}	42 ± 3^{aA}
LPS	45 ± 3^{aA}	56 ± 8^{bA}	61 ± 16^{bA}	53 ± 4^{bB}
LPS + SS	41 ± 2^{aA}	45 ± 8^{aA}	46 ± 4^{aA}	45 ± 3^{aB}
Platelet count, $\times 10^6$/mL				
Control	355 ± 70^{aA}	288 ± 50^{aA}	282 ± 65^{aA}	293 ± 76^{aA}
SS	284 ± 31^{aA}	286 ± 35^{aA}	286 ± 24^{aA}	300 ± 19^{aA}
LPS	272 ± 25^{aA}	143 ± 34^{bB}	244 ± 72^{aA}	237 ± 87^{aA}
LPS + SS	281 ± 46^{aA}	249 ± 13^{aB}	237 ± 48^{aA}	234 ± 68^{aA}

Control: Healthy dogs, SS: SAMe and silybin, LPS: lipopolysaccharide, LPS + SS: lipopolysaccharide + SAMe and silybin.
a, b, c: Different letters show statistically significant changes within groups, at least $P < 0.05$.
A, B: Different letters show statistically significant changes between groups, $P < 0.05 - P < 0.01$.

counts, and hematocrit value (25). In a previous study (25), total WBC counts were found to have a lower specificity (65%) and sensitivity (52%) to estimate sepsis severity when compared with those (61% and 91%, respectively) of serum CRP levels in dogs with parvoviral enteritis. Thus, in the present study, it is difficult to decide if LPS-induced inflammation was limited or inhibited by SAMe and silybin treatment based on the total WBC count alone. However, LPS-associated thrombocytopenia was not observed in dogs treated with SAMe and silybin, probably because of which SAMe or silybin or both inhibited platelet aggregation and excessive consumption, which are defined as a sign of DIC in septic patients (8,16) and dogs with endotoxemia (3,5,21,23). SAMe and silybin administration inhibited LPS-associated hematocrit elevation for 24 h, meaning that this treatment appears to be effective to prevent dehydration, most probably due to vascular permeability protection and inhibition of some clinical signs such as diarrhea leading to excessive fluid losses from the body in the course of endotoxemia (26). Released vasoactive substances such as TNF-α, histamine, and leukotrienes in response to LPS lead to vascular permeability disorder, giving rise to the plasma losses from intravascular space into interstitial space and hemoconcentration (26). Thus, SAMe- and silybin-related vascular permeability protection may result from vasoactive mediator inhibitions.

Hepatorenal injury was characterized by increasing serum ALT, ALP, BUN, and Cr levels after LPS administration (Table 3). Our observations expanded the findings of the previous study, which was performed in vitro on canine hepatocytes (18), by demonstrating the protection of both liver and renal injuries in response to LPS in in vivo conditions. A possible mechanism is that SAMe and silybin in combination could inhibit the inflammation and oxidative stress that are associated with liver injury and development of liver diseases (18). Our previous studies showed that oxidative stress inhibition by choline treatment resulted in decreased sepsis severity and good prognosis in dogs with endotoxemia (27).

These positive effects of SAMe and silybin may be associated with attenuated IL-1β-induced inflammation and oxidative stress, as well as reduced cytokine-induced PGE2, IL-8, and macrophage chemotactic protein-1 production (18). Another possible mechanism may be that SAMe and its metabolite methylthioadenosine inhibit LPS-induced TNF-α expression by blocking the binding of LPS to histone-3, a TNF-α promoter (28), and increase

Table 3. Some serum biochemical findings before (baseline) and after the treatments in all groups, each of which consisted of five dogs.

Parameters	Baseline	1 h	4 h	24 h
ALT, IU				
Control	21 ± 8^{aA}	23 ± 8^{aA}	22 ± 11^{aA}	21 ± 4^{aA}
SS	29 ± 6^{aA}	29 ± 4^{aA}	28 ± 3^{aA}	27 ± 5^{aA}
LPS	31 ± 19^{aA}	57 ± 22^{bB}	193 ± 150^{bB}	76 ± 24^{aB}
LPS + SS	$3\,4 \pm 7^{aA}$	34 ± 8^{aAB}	61 ± 20^{bB}	56 ± 18^{abAB}
ALP, IU				
Control	43 ± 7^{aA}	51 ± 11^{aA}	42 ± 5^{aA}	43 ± 11^{aA}
SS	47 ± 16^{aA}	45 ± 19^{aA}	44 ± 13^{aA}	45 ± 16^{aA}
LPS	40 ± 24^{aA}	100 ± 74^{bB}	174 ± 137^{abA}	170 ± 63^{abA}
LPS + SS	43 ± 28^{aA}	43 ± 31^{aA}	43 ± 24^{aA}	47 ± 9^{aA}
BUN, mg/dL				
Control	14 ± 4^{aA}	15 ± 4^{aA}	17 ± 7^{aA}	13 ± 3^{aA}
SS	15 ± 2^{aA}	14 ± 3^{aA}	14 ± 4^{aA}	13 ± 2^{aA}
LPS	18 ± 3^{aA}	32 ± 8^{bB}	38 ± 16^{bA}	55 ± 10^{bB}
LPS + SS	13 ± 4^{aA}	14 ± 3^{aA}	14 ± 4^{aA}	15 ± 6^{aA}
Cr, mg/dL				
Control	0.9 ± 0.1^{aA}	1.0 ± 0.0^{aA}	0.9 ± 0.2^{aA}	0.9 ± 0.0^{aA}
SS	1.3 ± 0.1^{aA}	1.1 ± 0.1^{aA}	1.4 ± 0.5^{aA}	1.7 ± 0.2^{aB}
LPS	0.9 ± 0.0^{aA}	1.5 ± 0.2^{bB}	1.4 ± 0.2^{bA}	1.7 ± 0.2^{bB}
LPS + SS	1.4 ± 0.2^{aA}	1.1 ± 0.1^{aA}	1.4 ± 0.5^{aA}	1.1 ± 0.2^{aA}

Control: Healthy dogs, SS: SAMe and silybin, LPS: lipopolysaccharide, LPS + SS: lipopolysaccharide + SAMe and silybin.
a, b: Different letters showed statistically significant changes within groups, at least $P < 0.05$.
A, B: Different letters showed statistically significant changes between groups, $P < 0.05 - P < 0.01$.

liver levels of glutathione, an important compound for liver health (19). Gonzalez-Corea et al. (29) reported that increase in glutathione levels and activity might be the main element in the hepatoprotective action of exogenous SAMe administration. Silybin was also thought to be hepatoprotective by several mechanisms, including activity against lipid peroxidation, cytochrome P450 inhibition, and the inhibition of transformation of stellate hepatocytes into myofibroblasts that lead to cirrhosis (20). Turgut et al. (30) reported that silymarin showed antioxidant and protective effects on renal injury, as well. Based on our study design, it is not possible to discriminate whether one of them (SAMe or silybin) is better to create these positive effects than the other.

LPS-associated cytokine increases (TNF-α and interleukins) lead to production and release of hepatic acute-phase proteins into circulation, and this process results in hepatic injury and coagulation disorders (5,8,16). The presence of hemostasis abnormalities was shown by the prolonged global clotting times (PT and aPTT) in natural cases of dogs with sepsis (8,23). In this study, the coagulation cascade was evaluated by use of TEG, because TEG results have a higher negative or positive predictive value in identifying bleeding dogs than PT, aPTT and D-dimer, which are widely used in veterinary medicine (16). TEG provides valuable information of hemostasis by measuring graphically and numerically the clotting time, clot firmness, and fibrinolysis of the patients (9,10,12,22).

In this study, although there were differences of baseline R values between the control and SS/LPS groups, observed TEG parameters before treatments (Table 4) were within the reference limits reported for dogs (9,10). These differences may be due to biological variations of TEG parameters. A study investigating the biological variation of coagulation assays in clinically healthy dogs showed that PT, aPTT, and D-dimer levels showed a degree of individuality, which makes the use of population-based reference ranges alone an insensitive

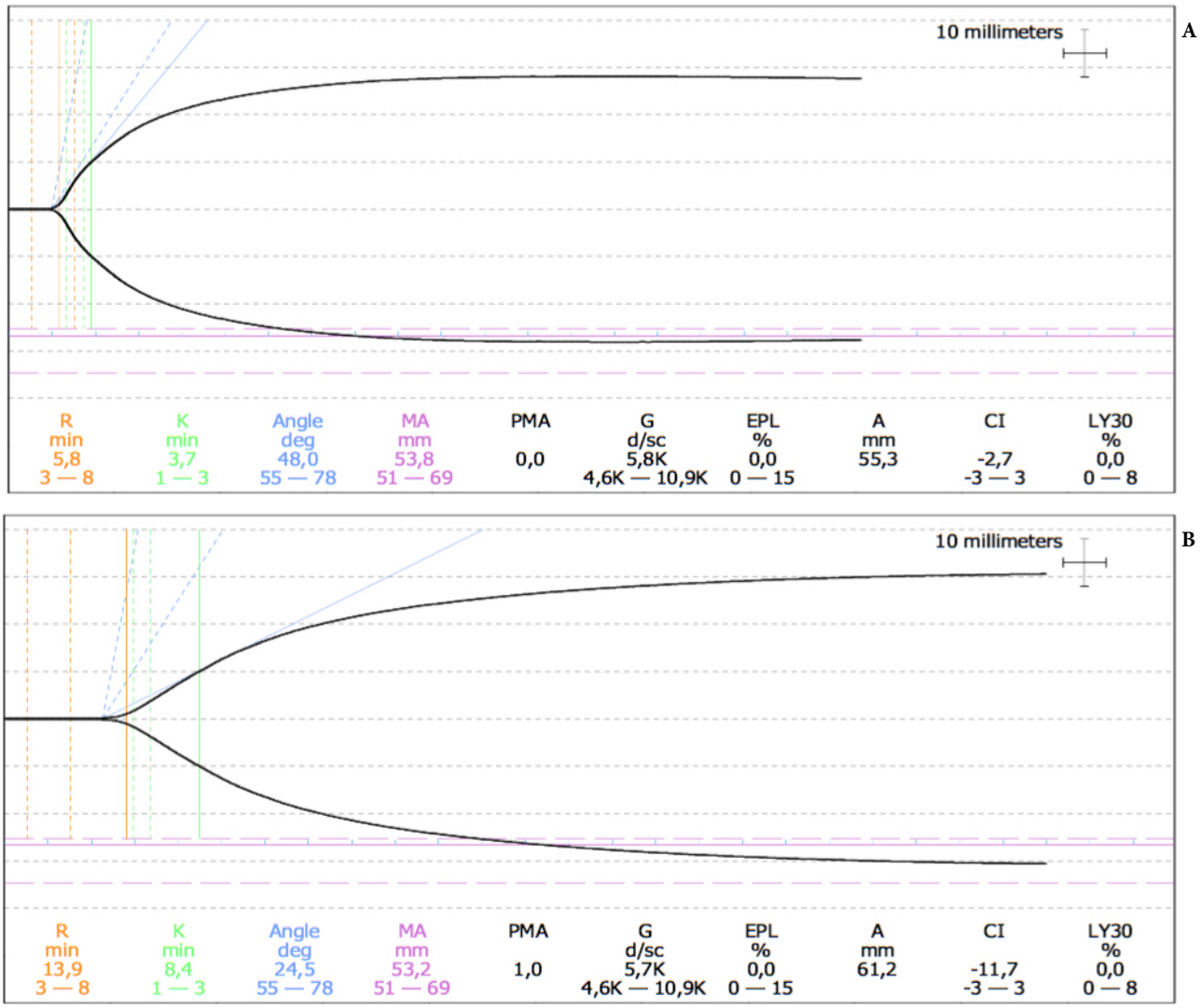

Figure 1. Thromboelastographic (TEG) records before (A) and 4 h after (B) LPS administration in a dog. A) TEG parameters are in the reference range (normocoagulable). B) Prolonged R and K times and decreased α-angle indicate a hypocoagulable state in response to LPS.

interpretation criterion, whereas a population-based reference interval seems to be sensitive for interpreting all TEG parameters (9). Also, in this study, male dogs were selected for experiments to avoid a possible effect of sex on TEG measurements, in accordance with a previous study (10).

In contrast to the control and SS groups, some TEG parameters changed significantly during the study in the LPS group. In the present study, observed increases in R and K times along with a decrease in α-angle within 4 h after LPS injection were compatible with a hypocoagulable state (9). This is in line with a previous study showing that, following LPS administration, TEG measurements revealed a concurrent decrease in MA and increase in R time (9). These results showed that LPS administered at a sublethal dose caused hemostasis alterations in the early phase of endotoxemia in dogs. Since R and K times are primarily influenced by plasma clotting factors, fibrinogen plasma concentration, and platelet count, we may speculate that prolonged R and K times were associated with decreased plasma clotting factor and/or decreased platelet count (consumptive coagulopathy), indicating a hypocoagulable state in response to LPS (Figures 1A and 1B). Our explanations are consistent with the previous study results, including that plasma clotting factors (FII–FXIII) (5) and plasma natural anticoagulants (antithrombin, protein C, and protein S) decreased after LPS administration in dogs (9).

In this study, while R and K times increased, platelet count decreased in the early phase of endotoxemia in dogs,

Table 4. Selected TEG measurements before (baseline) and after the treatments in all groups, each of which consisted of five dogs.

Parameters (reference)	Baseline	1 h	4 h	24 h
R time, min (1.8–8.6 min)				
Control	2.1 ± 0.0^{aA}	2.2 ± 0.1^{aA}	1.9 ± 0.6^{aA}	2.6 ± 0.9^{aA}
SS	4.3 ± 0.7^{aB}	4.4 ± 0.6^{aB}	4.4 ± 1.3^{aA}	4.2 ± 0.4^{aA}
LPS	5.1 ± 0.7^{aB}	6.2 ± 1.2^{aB}	8.1 ± 4.0^{aB}	9.9 ± 0.8^{bB}
LPS + SS	3.3 ± 1.6^{aAB}	4.2 ± 0.7^{aB}	4.6 ± 0.9^{aB}	3.9 ± 0.5^{aA}
K time, min (1.3–5.7 min)				
Control	1.1 ± 0.4^{aA}	1.6± 0.2^{aA}	1.0 ± 0.4^{aA}	1.2 ± 0.1^{aA}
SS	1.8 ± 0.3^{aA}	2.6±1.3^{aAB}	2.0 ± 1.0^{aA}	1.5 ± 0.4^{aA}
LPS	2.0 ± 0.2^{aA}	4.3± 1.2^{bB}	4.4 ± 0.8^{bB}	5.4 ± 0.6^{bB}
LPS + SS	1.9 ± 0.4^{aA}	2.1±0.3^{aA}	2.2 ± 0.4^{aA}	2.0 ± 1.0^{aA}
α-angle, degrees (36.9–74.6 degrees)				
Control	65 ± 5^{aA}	71 ± 11^{aA}	72 ± 10^{aA}	68 ± 7^{aA}
SS	64 ± 8^{aA}	55 ± 14^{aAB}	58 ± 12^{aAB}	68 ± 9^{aA}
LPS	63 ± 11^{aA}	47 ± 12^{bB}	50 ± 5^{abB}	34 ± 6^{bB}
LPS + SS	65 ± 3^{aA}	62 ± 7^{aAB}	65 ± 8^{aAB}	64 ± 10^{aA}
MA, mm (42.9–67.9 mm)				
Control	63 ± 6^{aA}	61 ± 8^{aA}	62 ±11^{aA}	60±7^{aA}
SS	64 ± 7^{aA}	65 ± 9^{aA}	64 ±7^{aA}	63±10^{aA}
LPS	61 ± 5^{aA}	71 ± 7^{aA}	70 ±6^{aA}	74±8^{aA}
LPS + SS	62 ± 4^{aA}	74 ± 6^{aA}	75±7^{aA}	78±4^{aA}
G value, kdyn/cm^2 (3.2–9.6 kdyn/cm^2)				
Control	7 ± 2^{aA}	7 ± 1^{aA}	7 ± 3^{aA}	6 ± 1^{aA}
SS	5 ± 1^{aA}	5 ± 2^{aA}	5 ± 1^{aA}	5 ± 0^{aA}
LPS	6 ± 0^{aA}	6 ± 0^{aA}	7 ± 1^{aA}	7 ± 0^{aA}
LPS + SS	6 ± 0^{aA}	6 ± 1^{aA}	6 ± 0^{aA}	5 ± 1^{aA}

Control: Healthy dogs, SS: SAMe and silybin, LPS: lipopolysaccharide, LPS + SS: lipopolysaccharide + SAMe and silybin.
a, b: Different letters between groups show statistically significant changes within groups, at least $P < 0.05$.
A, B: Different letters show statistically significant changes between groups, $P < 0.05 - P < 0.01$.

probably indicating an inverse correlation between them and platelet dysfunction, as reported earlier (9). Canine experimental endotoxemia can cause the abnormalities of primary (21), secondary, and tertiary hemostasis (5,9). Following LPS administration, MA and G values did not change, showing that the severity of endotoxemia in the present study was not enough to impair the peak clot rigidity and/or fibrinolysis in dogs (9). SAMe and silybin treatment attenuated these coagulation responses in dogs with LPS, probably regulated by circulating cytokines such as TNF-α (18,28) and platelet responses to LPS. The modulation by SAMe of the inflammatory response triggered by LPS is a crucial part of its hepatoprotective effect. Hepatoprotective effects of SAMe and silybin in combination may also improve the production and synthesis of coagulant (FI, II, etc.) and anticoagulant factors such as antithrombin in dogs with endotoxemia (9).

In summary, this study suggests that SAMe and silybin administration may be beneficial by regulating the coagulation cascade and protecting hepatorenal function in dogs with endotoxemia. Improving hemostatic

abnormalities of this combination has not been described previously and is important in understanding its therapeutic effect.

Acknowledgment
This study was supported in part by the Scientific and Technological Research Council of Turkey (TÜBİTAK) – Project ID: TOVAG - 109O660.

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